MAIL STOP AMENDMENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

ARIEL G. NOTCOVICH et al — Group Art Unit: 1641

Application No.: 10/578,860 — Examiner: LAM, ANN Y

Filed: June 30, 2006 — Docket No.: 27396U

For: SYSTEM AND METHOD FOR CARRYING OUT MULTIPLE BINDING REACTIONS IN AN ARRAY FORMAT

DECLARATION UNDER 37 C.F.R. §1.132

I, Professor Gideon Schreiber, a citizen of Israel, hereby declare and state:

1. I am an Associate Professor at the Department of Biological Chemistry at the Weizmann Institute of Science. A list of my publications is attached as Appendix A together with my Curriculum Vitae, attached as Appendix B.

2. I have over 20 years of experience in research of protein-protein interactions, protein design and structural proteomics.

3. I have a vast academic and professional experience with respect to the technology at hand, including the Biacore and BioRad biosensor platforms (see for example References 3, 7, 8, 10, 11, 12, 13, 16, 19, 24, 28, 29, 31, 32, 37, 38, 40, 44, 49, 50, 54, 55, 56, 57 and 58 in the attached Appendix A).

4. I am not being compensated for my work in connection with this Declaration.

5. For the purpose of my analysis I specifically reviewed in detail the Official Action dated August 6, 2009 as well as the following publications:

Malmqvist et al., US Patent No. 6,200,814, issued March 13, 2001 (henceforth: Malmqvist et al);

Newgard et al., US Patent No. 6,110,707, issued Aug 29, 2000;

Siddigi et al., US Patent No. 5,541,113, issued July 30, 1996;

Lambert, J., US Publication No. 2006/0210984, published September 21, 2006;

Karlsson et al., US Publication 2005/0014179, published January 20, 2005;

Karlsson et al., priority documents, US provisional Application No. 60/477,909, filed on June 6, 2003 and US Provisional Application No. 60/526364, filed 1/12/2003;

Kansa et al., US Patent No. 6,478,839, issued November 12, 2002;

Natesan et al., US Publication No. 2002/0048792;

Rich et al., Higher-throughput, label-free, real-time molecular interaction analysis, Analytical Biochemistry 361 (2007) 1–6.

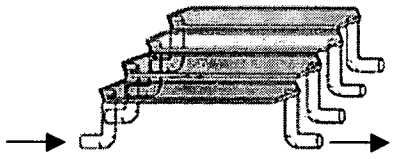
6. The state of the art prior to the filing of US Application No.:10/578,860, herein referred to as the present application, will initially be discussed and explained.

7. Dynamic information about molecular interaction analysis has become an essential component of life-science research and the pharmaceutical industry. Over the past 15 years, interaction technology at the molecular level has grown considerably with the gradual development and introduction of a number of instruments or flow cells.

8. In short, around 1990, Pharmacia Biosensor released the first commercially viable optical biosensor platform also known as "Biacore." This platform was an important landmark because of its combination of micro fluidic and robotic technologies. Magnus *Malmqvist*, Robert *Karlsson* and Inger *Rönnberg* are known employees of Biacore, Uppsala, Sweden.

9. The first-edition instrument had four flow cells, but each of the cells could only be addressed separately and individually as shown in the bellow Figure 1:

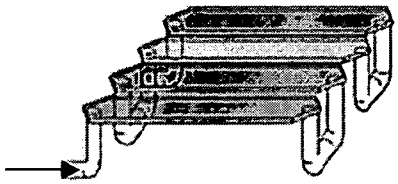
Figure 1



As shown in Figure 1, one analyte could be studied over one target at a time (one × one). Typically, the same sample or target species would be tested over the target surface and then over a reference surface in a separate injection. Although this was a substantial burden, the instrument was fully automated and capable of overnight unattended *serial* operation i.e., not simultaneous.

10. In the late 1990s Biacore AB (former biosensor division of Pharmacia) developed a new platform called "Biacore^{TM} 2000", the major advance in the technology was a micro fluidic structure which made it possible for the user to analyze *one* analyte sample (or one target specie) over *four* flow cell surfaces separately or in series ("one × several" approach). Thus, it was possible to immobilize separate ligands and then inject one analyte sample over the separate immobilized ligands.

11. Data was collected from a reference surface (one flow cell was used as a reference) and three reaction surfaces at the same time (other 3 flow cells). Figure 2 below describes the Biacore™ 2000 (schematic drawings taken from Rebecca L. Rich, David G. Myszka, Higher-throughput, label-free, real-time molecular interaction analysis, Analytical Biochemistry 361 (2007) 1–6).

Figure 

12. The Biacore™ 3000 system was an improvement over the Biacore™ 2000 system. The Biacore™ 3000 system related to the applicability of highly refractive index buffers. For the determination of a kinetic parameter, the Biacore™ 2000 and Biacore™ 3000 systems required multiple runs with a regeneration step after each run, i.e., after each binding assay was performed. Analysis of a plurality of analytes or analyte concentrations necessitated *serial* operation and thus required regeneration. It should be stressed that the step of regenerating the sensitizing spot is harmful to the immobilized ligand.

13. Malmqvist et al. essentially relates to the technology of the original Biacore™ 2000 and Biacore™ 3000 systems while introducing several improvements to the fluid delivery system, as I will further explain in detail below. In Malmqvist et al. the fluid delivery system is based on laminar flow later named "hydrodynamic addressing".

14. Malmqvist et al. is aimed at improving the sample delivery in SPR systems. In terms of kinetic analysis, the authors remain in the field of the "one × several" approach (mentioned above) which adheres to a paradigm that existed at the time; that is, analysis of *one* analyte concentration over *several* flow cell surfaces or sensitization spots.

15. Malmqvist et al. only propose a gradient with regard to the amount of immobilized ligand bound to the surface of each sensing area, where the gradient is in fact a byproduct of laminar flow [Malmqvist et al. col. 13 line 12 – col. 14 line 37, in particular col. 14 lines 26-37]. The gradient of the immobilized ligand density is not a parameter which is used in the calculation of the kinetic parameters. The kinetic parameters, such as k_a and k_d, are derived from the binding of the analyte at varying concentrations with the immobilized ligand regardless of immobilized ligand density.

While Malmqvist et al. propose a gradient of *bound ligand*, it does not disclose a binding assay involving a plurality of *analyte* concentrations (being the second binding member in the subject application). Beyond adhering to the existing concept, there are also technological reasons for that. The laminar flow (hydrodynamic addressing) method as presented by Malmqvist et al. necessitates different flow rates to create a boundary between the flown solutions. Different flow rate might change the kinetic behavior of the interaction, and thus obtaining a full and accurate binding assay with a plurality of analyte concentrations is practically impossible with this technology.

16. Malmqvist et al. propose several theoretical options of making sensitized matrices using a 2D laminar flow cell (col. 15 line 37 – col. 16 line 27, Fig. 13), applying different molecules, e.g. different oligonucleotides. The option of presenting a second binding member (the binding member to the immobilized ligand) **at a plurality of concentrations** is not described by Malmqvist et al. nor is it possible as detailed above (see point 15 above).

17. The "one × several" approach was the paradigm before and during 2004; but, also at the time Bio-Rad's ProteOn XPR36 was released. The "one × several" approach was the paradigm also adhered to by Biacore systems Biacore™ 2000 and Biacore™ 3000, but also the later systems S51 (released on 2001) using the "Y-shaped" flow cells (Figure 3) and the T100 having the "U" shaped flow cells, released on 2005 (Figure 4).

Figure 3

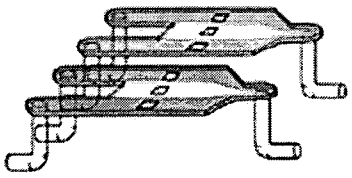
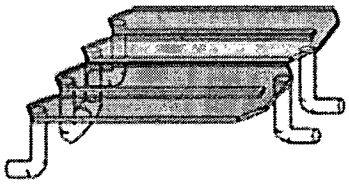


Figure 4



18. These Biacore instruments accommodated analysis of samples from one 96- or 384-well plate while processing one sample at a time i.e. serial analyte processing. Malmqvist et al. do not disclose or hint otherwise. Throughout the entire text of Malmqvist et al. and all of its respective examples a single analyte concentration is run at a time. Analysis of two or more e.g. plurality of analyte concentrations, would require a serial processing and thus necessitate regeneration step.

19. The serial analyte processing of Malmqvist et al. cannot simultaneously obtain one or more kinetic parameters. Accurate determination of kinetic parameters necessitates a plurality of single-reaction processing of several analyte concentrations, which in the flow cells described in Malmqvist et al. cannot be performed simultaneously. As a corollary, Malmqvist et al. neither describe simultaneously obtaining a kinetic parameter nor describe any data indicative of a binding reaction between a first (the immobilized ligand) and a second binding members (plurality of *analyte* concentrations) at the spots. The outcome of Malmqvist et al. must, therefore, be that for a binding essay involving a plurality of analyte concentrations a serial analyte processing must be required and cannot be avoided i.e. a simultaneous analysis of a plurality of target species of different concentrations cannot be obtained when utilizing the system of Malmqvist et al.

20. Lambert does not add a technical contribution to Malmqvist in this respect. In Lambert a single analyte sample is run at a time. Lambert merely describes simultaneous binding measurements *in general* but not simultaneously obtaining a kinetic parameter which requires simultaneous processing of several analyte concentrations, as presently claimed.

21. Malmqvist et al. do not teach how to avoid a regeneration step. A regeneration step was a major drawback of the technology at the time and is not a trivial matter that could be understood by default. Major players in the field of molecular interaction analysis invested money and research time to develop a technology that would not necessitate regeneration.

22. Detailed review of analysis formats, approaches and instruments may be found in Rebecca L. Rich, David G. Myszka, Higher-throughput, label-free, real-time molecular interaction analysis (Analytical Biochemistry 361 (2007) 1–6).

23. According to the technology which existed prior to the development of BioRad technologies, in part, the subject matter described in the present application, prior to the contacting of the sensor surface with a sample of a new analyte concentration, the ligand surface had to be restored or "regenerated" by treating the surface with a regeneration solution. I have reviewed Malmqvist et al., Newgard et al. and Lambert. The silence of these documents with respect to the necessity of regeneration should be attributed solely to the fact that none of the technologies disclosed in these publications involved the processing of a plurality of analyte samples.

24. A later publication dated January 20, 2005 by Robert *Karlsson* (a Biacore researcher as Malmqvist) clearly states as follows:

"...***Before contacting the sensor surface with sample of a new analyte***

> ***concentration, the ligand surface is restored or "regenerated" by treating the surface with a regeneration solution capable of removing any bound analyte while not destroying the ligand.*** In that way, all the different samples will contact essentially one and the same ligand surface as far as ligand density is concerned. The association and dissociation rate constants can then be obtained from the collected binding data by fitting the data to mathematical descriptions of interaction models in the form of differential equations. Usually, the binding data for all the samples are used in the same fit, a procedure referred to as global fitting. From the determined association and dissociation rate constants k_A and k_d, the equilibrium constant, K_D, and the affinity constant K_A (K_A =1/ K_D) of the interaction can in turn be calculated." [Karlsson et al., page 1, paragraph 6]
>
> "...***From the prior art it may therefore be concluded*** *that for determining kinetic rates for molecular interactions using systems based on biosensors and affinity analysis,* ***it is necessary to regenerate the immobilized ligand prior to contacting the sensor surface with a different concentration of analyte to thereby present essentially one and the same ligand surface to each analyte concentration,*** unless (i) a continuous gradient of the analyte is used, or (ii) initial binding rates are determined in systems free from mass transport limitations.. [0016] It is an object of the present invention to provide a sensor-based method for determining chemical interaction parameters, including kinetic rate constants, by stepwise titration, which method obviates regeneration procedures while permitting measurements under mass transport limitation." [Karlsson et al., page 1, paragraphs 15-16]

Therefore, the prior art before and around January 20, 2005 and certainly before November 11, 2003 necessitated regeneration of the surface. The silence of Malmqvist et al. with respect to a regeneration step is attributed to the fact that it did not even remotely attempt to solve this problem but focused on providing improved selective ligand immobilization techniques (or sensitization) on the basis of the laminar flow technology.

25. Natesan et al. are also a mere general description of determining binding affinity/dissociation constant. Natesan et al. do not describe simultaneously obtaining a kinetic parameter.

26. It should be stated that the gradient of immobilized ligand as a result of laminar flow in general and specifically as a function of time and distance of the sensing spot (as Malmqvist et al.) is technically incompatible and certainly insufficient infrastructure for simultaneously obtaining a kinetic parameter, as already explained in details above.

27. The present Applicant describes, for the first time, a system and method for the determination of kinetic parameters of a binding reaction, referred to in the application as "One-Shot Kinetics" (OSK). This method allows carrying out a plurality of binding reactions without the need of a regeneration step and without the need for repeated experiments which

are known to harm the 'probe' or the immobilized ligands. The system and method of Applicants' claimed subject matter allow simultaneous binding reactions of a plurality of analyte concentrations with one or several probe densities. The plurality of reactions is monitored simultaneously and signals indicative of the binding reactions are obtained and analyzed so as to produce a kinetic measurement of the binding.

28. An additional advantage, apart from the avoidance of a regeneration step, of the simultaneous reaction of a plurality of analyte combinations, is in reducing time and temperature fluctuations which otherwise increase if separate or serial reactions are performed and processed for each particular analyte sample.

29. The One-Shot Kinetics provides a solution to the lack of high throughput technology which enables real-time, label-free monitoring of kinetics of multiple bio-molecular interactions involving plurality of analyte concentration combinations all at the same "shot". Neither the above mentioned Biacore instruments nor Malmqvist et al. demonstrate the present technology. The first named inventor, Notcovich, together with major research figures in the field have reported on the One-Shot Kinetics in a 2006 publication which I also reviewed. From this publication, I wish to quote the following passage:

> "..One of the first applications we wanted to explore was the ability to collect kinetic data for six different concentrations of analyte at the same time. Historically, response data for different analyte samples have been collected sequentially. ***Parallel collection of different analyte concentrations could improve sample throughput and also render obsolete the need to regenerate the target sensor surface***. To test the throughput and performance of the ProteOn instrument, we selected a well-characterized small molecule interaction system, namely the binding of sulfonamide-based inhibitors to the enzyme carbonic anhydrase II. The enzyme was coupled at different densities within the six flow channels using amine chemistry. We found that response data collected simultaneously for six concentrations of each compound could be globally fit to a simple interaction model. The affinities determined from the ProteOn instrument correlated well with those determined using titration calorimetry and Biacore. The application of "one-shot" kinetics and the ability of the ProteOn instrument to collect kinetic data for small molecule interactions could

significantly expand the speed and application of optical biosensor technology in drug discovery."

30. Further review of the technological advantages of the OSK technology may be found in Rebecca L. Rich and David G. Myszka at the bottom page 4, last paragraph, where it is stated that the technology allows for "... generating a full analyte concentration series with one injection [25,26]. This reduces analysis time significantly and eliminates the need for surface regeneration."

31. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: 27/01/2010

Prof. Gideon Schreiber

Appendix A

Prof. Gideon Schreiber - Publications

1. **Kiel C, Filchtinski D, Spoerner M, Schreiber G, Kalbitzer HR, Herrmann C.** Improved binding of raf to Ras.GDP is correlated with biological activity. J Biol Chem. 2009 **284:** 31893-902 .

2. **Rahat O, Alon U, Levy Y, Schreiber G.** Understanding hydrogen-bond patterns in proteins using network motifs. Bioinformatics 2009 **25:** 2921-8 .

3. **Phillip Y, Sherman E, Haran G, Schreiber G.** Common Crowding Agents Have Only a Small Effect on Protein-Protein Interactions. Biophysical J. 2009 **97:** 875-85 .

4. **Cohen M, Potapov V, Schreiber G.** Four Distances between Pairs of Amino Acids Provide a Precise Description of their Interaction. PLOS Comp. 2009 **ie1000470:** .

5. **Moraga I, Harari D, Schreiber G, Uzé G, Pellegrini S.** Receptor density is key to the interferon alpha2/beta differential activities. Mol Cell Biol. 2009. **29:**4778-87 .

6. **Potapov V, Cohen M, Schreiber G.** Assessing computational methods for predicting protein stability upon mutation: good on average but not in the details. PEDS. 2009. **22:** 553-60 .

7. **Cohavi O, Tobi D, Schreiber G.** Docking of antizyme to ornithine decarboxylase and antizyme inhibitor using experimental mutant and double-mutant cycle data. J.Mol.Biol., 2009. **390:** 503-15 .

8. **Harel, M., Spaar, A. & Schreiber, G.** Fruitful and futile encounters along the association reaction between proteins. Biophys J., 2009. **96:** 4237-48 .

9. **Schreiber, G., Haran, G. & Zhou, H. X.** Fundamental Aspects of Protein-Protein Association Kinetics. Chem Rev, 2009. **109:** 839-860 .

10. **Bravman, T., Bronnera, V., Nahshol, O. & Schreiber, G.** The ProteOn XPR36™ Array System – High Throughput Kinetic Binding Analysis of Biomolecular Interactions. Cellular and Molecular Bioengineering. Cellular and Molecular Bioengineering, 2008 **1:** 216-228 .

11. **Kalie E, Jaitin DA, Podoplelova Y, Piehler J, Schreiber G.** The stability of the ternary interferon-receptor complex rather than the affinity to the individual subunits dictates differential biological activities. J Biol Chem., 2008. **283:**32925-32936 .

12. **Potapov V., Reichmann D., Abramovich R., Filchtinski D., Zohar N., Ben Halevy D., Edelman M. Sobolev V., and Schreiber G.** Computational redesign of a protein-protein interface for high affinity and binding specificity using modular architecture and naturally occurring template fragments. J. Mol. Biol, 2008. **384:** 109-119 .

13. **Manjing Pan, Eyal Kalie, Brian J. Scaglione, Elizabeth S. Raveche, Gideon Schreiber Jerome A. Langer.** Mutation of the IFNAR-1 Receptor Binding Site of Human IFN-a2 Generates Type I IFN Competitive Antagonists. Biochemistry, 2008. **47:** 1051-1060 .

14. **Cohen, M., Reichmann, D., and Schreiber, G.** Similar chemistry, but different bond preferences for inter versus intra-protein interactions. Proteins, 2008. **72:** 741-53 .

15. **Rahat, O., Yitzhaky, A., and Schreiber, G.** Cluster conservation as a novel tool for studying protein-protein interactions evolution. Proteins, 2008. **71:** 621-30 .

16. **Reichmann, D., Phillip, Y., and Schreiber, G.** On the contribution of water-mediated interactions to protein-complex stability. Biochemistry, 2008. **47:** 1051-60 .

17. **Uze, G., Schreiber, G., Piehler, J., and Pellegrini, S.** The receptor of the type I interferon family. Curr Top Microbiol Immunol. 2007 **316:** 71-95 .

18. **Adam, Y., Tayer, N., Rotem, D., Schreiber, G., and Schuldiner, S.** The fast release of sticky protons: kinetics of substrate binding and proton release in a multidrug transporter. Proc Natl Acad Sci U S A, 2007. **104:** p.17989-17994 .

19. **Reichel, A., Schaible, D., Al Furoukh, N., Cohen, M., Schreiber, G., and Piehler, J** Noncovalent, site-specific biotinylation of histidine-tagged proteins. Anal Chem, 2007. **79:** p.8590-8600 .

20. **Neuvirth, H., Heinemann, U., Birnbaum, D., Tishby, N., and Schreiber, G.** ProMateus--an open research approach to protein-binding sites analysis. Nucleic Acids Res., 2007. **35:** W543-8 .

21. **Harel, M., Cohen, M. & Schreiber, G.** On the Dynamic Nature of the Transition State for Protein-Protein Association as Determined by Double-mutant Cycle Analysis and Simulation. J Mol. Biol., 2007. **371:** p.180-96 .

22. **Hodis, E., Schreiber, G., Rother, K. & Sussman, J.L.** eMovie: A storyboard-based tool for making molecular movies. TIBS, 2007. **32:** p.199-204 .

23. **Jaitin, D. A. & Schreiber, G.** Upregulation of a Small Subset of Genes Drives Type I Interferon-Induced Antiviral Memory. J Interferon Cytokine Res. 2007 **27** p.653-664 .

24. **Kalie, E., Jaitin, D. A., Abramovich, R. & Schreiber, G.** An IFNalpha 2 mutant optimized by phage-display for IFNAR1 binding confers specifically enhanced antitumor activities. J Biol Chem., 2007. **282:** p.11602-11 .

25. **Reichmann, D., Rahat, O., Cohen, M., Neuvirth, H., & Schreiber, G.** The molecular architecture of protein-protein binding sites. Curr Opin Struct Biol., 2007. **17:** p. 67-76.

26. **Raveh, B., Rahat, O., Basri, R.& Schreiber, G.** Rediscovering secondary

structures as network motifs--an unsupervised learning approach. Bioinformatics, 2007. **23:** p. e163-169.

27. **Kozer, N., Kuttner, Y.Y., Haran, G., & Schreiber, G.** Protein-Protein Association in Polymer Solutions: From Dilute to Semidilute to Concentrated. Biophysical Journal, 2007. **92:** p. 2139-2149.

28. **Reichmann, D., Cohen, M., Abramovich, R., Dym, O., Lim, D., Strynadka, JNC., & Schreiber, G.** A Binding hot spots in the TEM1-BLIP interface in light of its modular architecture. J Mol Biol., 2007. **365:** p. 663-679.

29. **Schmeisser, H., Kontsek, P., Esposito, D., Gillette, W., Schreiber, G., & Zoon, K. C.** Binding Characteristics of IFN-alpha Subvariants to IFNAR2-EC and Influence of the 6-Histidine Tag. J Interferon Cytokine Res., 2006. **26:** p. 866-876.

30. **Schreiber G, Shaul Y, Kottschalk KE** Electrostatic design of protein-protein association rates. Methods Mol Biol 2006. **340:** p. 235-49.

31. **Slutzki, M, Jaitin DA, Ben Yehezker, T, Schreiber G.** Variations in the unstructured C-terminal tail of interferons contribute to differential receptor binding and bilogical activity. J Mol Biol 2006. **360:** p. 119-30.

32. **Jaitin DA, Roisman LC, Jaks E, Gavutis M, Piehler J, Van der Heyden J, Uze G, Schreiber G.** Inquiring into the Differential Action of Interferons (IFNs): an IFN-{alpha}2 Mutant with Enhanced Affinity to IFNAR1 Is Functionally Similar to IFN-{beta}. Mol Cell Biol 2006. **26:** p. 1988-97.

33. **Schreiber G.** The nitty-gritty of protein interactions. Structure 2005. **13:** p. 1737-8.

34. **Kuttner YY, Kozer N, Segal E, Schreiber G, Haran G.** Separating the contribution of translational and rotational diffusion to protein association. J Am Chem Soc 2005. **127:** p. 15138-44.

35. **Albeck S, Burstein Y, Dym O, Jacobovitch Y, Levi N, Meged R, Michael Y, Peleg Y, Prilusky J, Schreiber G, Silman I, Unger T, Sussman JL.** Three-dimensional structure determination of proteins related to human health in their functional context at The Israel Structural Proteomics Center (ISPC). This paper was presented at ICCBM10. Acta Crystallogr D Biol Crystallogr 2005. **61:** p. 1364-72.

36. **Shaul Y, Schreiber G.** Exploring the charge space of protein-protein association: A proteomic study. Proteins 2005. **60:** p. 341-52.

37. **Roisman LC, Jaitin DA, Baker DP, Schreiber G.** Exploring the charge space of protein-protein association: A proteomic study. J Mol Biol 2005. **353:** p. 271-81.

38. **Reichmann, D., Rahat, O., Albeck, S., Meged, R., Dym, O. & Schreiber, G.** A The modular architecture of protein-protein binding interfaces. Proc Natl Acad Sci U S A., 2005. **102:** p. 57-62.

39. **Gottschalk, K. E., Neuvirth, H. & Schreiber, G.** A novel method for scoring of docked protein complexes using predicted protein-protein binding sites. Protein Eng Des Sel. 2004. **17:** p. 183-189.

40. **Peleg-Shulman, T., Roisman, L. C., Zupkovitz, G. & Schreiber, G.** Optimizing the Binding Affinity of a Carrier Protein: A CASE STUDY ON THE INTERACTION BETWEEN SOLUBLE ifnar2 AND INTERFERON {beta}. J Biol Chem. 2004. **279:** p. 18046-18053.

41. **Kiel, C., Selzer, T., Shaul, Y., Schreiber, G. & Herrmann, C.** Electrostatically optimized Ras-binding Ral guanine dissociation stimulator mutants increase the rate of association by stabilizing the encounter complex. Proc Natl Acad Sci U S A. 2004. **101(25):** 9223-8.

42. **Kozer, N. & Schreiber, G.** Effect of Crowding on Protein-Protein Association Rates: Fundamental Differences between Low and High Mass Crowding Agents. Journal of Molecular Biology. 2004. **336:** p. 763-774.

43. **Neuvirth, H., Raz, R. & Schreiber, G.** ProMate: A Structure Based Prediction Program to Identify the Location of Protein-Protein Binding Sites. J Mol Biol. 2004. **338:** p. 181-199.

44. **Peleg-Shulman, Tsubery,T. Mironchik, M. Fridkin, M. Schreiber, G. and Shechter, Y.** Reversible PEGylation: A novel technology to release native interferon a2 over a prolonged time period. J. Med. Chem. **2004.** 279: p. 18046-18053.

45. **Chill JH, Quadt SR, Levy R, Schreiber G, Anglister J.** The human type I interferon receptor: NMR structure reveals the molecular basis of ligand binding. Structure. 2003. **11:** p. 791-802 .

46. **Chill, J.H., R. Nivasch, R. Levy, S. Albeck, G. Schreiber, and J. Anglister,** *The human interferon receptor: NMR-based modeling, mapping of the IFN-alpha 2 binding site, and observed ligand-induced tightening.* Biochemistry, 2002. **41**(11): p. 3575-85.

47. **Schreiber, G.,** *Kinetic studies of protein-protein interactions.* Curr Opin Struct Biol, 2002. **12**(1): p. 41-7.

48. **Frisch, C., A.R. Fersht, and G. Schreiber,** *Experimental assignment of the structure of the transition state for the association of barnase and barstar.* J Mol Biol, 2001. **308**(1): p. 69-77.

49. **Piehler, J. and G. Schreiber,** *Fast transient cytokine-receptor interactions monitored in real time by reflectometric interference spectroscopy.* Anal Biochem, 2001. **289**(2): p. 173-86.

50. **Roisman, L.C., J. Piehler, J.Y. Trosset, H.A. Scheraga, and G. Schreiber,** *Structure of the interferon-receptor complex determined by distance constraints from double-mutant cycles and flexible docking.* Proc Natl Acad Sci USA, 2001. **98**(23): p. 13231-6.

51. **Schreiber, G.,** *Methods for studying the interaction of barnase with its*

inhibitor barstar. Methods Mol Biol, 2001. **160**: p. 213-26.

52. **Selzer, T. and G. Schreiber,** *New insights into the mechanism of protein-protein association.* Proteins, 2001. **45**(3): p. 190-8.

53. **Shechter, Y., L. Preciado-Patt, G. Schreiber, and M. Fridkin,** *Prolonging the half-life of human interferon-alpha 2 in circulation: Design, preparation, and analysis of (2-sulfo-9-fluorenylmethoxycarbonyl)7- interferon-alpha 2.* Proc Natl Acad Sci USA, 2001. **98**(3): p. 1212-7.

54. **Albeck, S., R. Unger, and G. Schreiber,** *Evaluation of direct and cooperative contributions towards the strength of buried hydrogen bonds and salt bridges.* J Mol Biol, 2000. **298**(3): p. 503-20.

55. **Piehler, J., L.C. Roisman, and G. Schreiber,** *New structural and functional aspects of the type I interferon-receptor interaction revealed by comprehensive mutational analysis of the binding interface.* J Biol Chem, 2000. **275**(51): p. 40425-33.

56. **Selzer, T., S. Albeck, and G. Schreiber,** *Rational design of faster associating and tighter binding protein complexes.* Nat Struct Biol, 2000. 7(7): p. 537-41.

57. **Albeck, S. and G. Schreiber,** *Biophysical characterization of the interaction of the beta-lactamase TEM-1 with its protein inhibitor BLIP.* Biochemistry, 1999. **38**(1): p. 11-21.

58. **Piehler, J. and G. Schreiber,** *Biophysical analysis of the interaction of human ifnar2 expressed in E. coli with IFNalpha2.* J Mol Biol, 1999. **289**(1): p. 57-67.

59. **Piehler, J. and G. Schreiber,** *Mutational and structural analysis of the binding interface between type I interferons and their receptor Ifnar2.* J Mol Biol, 1999. **294**(1): p. 223-37.

60. **Selzer, T. and G. Schreiber,** *Predicting the rate enhancement of protein complex formation from the electrostatic energy of interaction.* J Mol Biol, 1999. **287**(2): p. 409-19.

61. **Vijayakumar, M., K.Y. Wong, G. Schreiber, A.R. Fersht, A. Szabo, and H.X. Zhou,** *Electrostatic enhancement of diffusion-controlled protein-protein association: comparison of theory and experiment on barnase and barstar.* J Mol Biol, 1998. **278**(5): p. 1015-24.

62. **Frisch, C., G. Schreiber, C.M. Johnson, and A.R. Fersht,** *Thermodynamics of the interaction of barnase and barstar: changes in free energy versus changes in enthalpy on mutation.* J Mol Biol, 1997. **267**(3): p. 696-706.

63. **Nolting, B., R. Golbik, J.L. Neira, A.S. Soler-Gonzalez, G. Schreiber, and A.R. Fersht,** *The folding pathway of a protein at high resolution from microseconds to seconds.* Proc. Natl Acad Sci USA, 1997. **94**(3): p. 826-30.

64. **Schreiber, G., C. Frisch, and A.R. Fersht,** *The role of Glu73 of barnase in catalysis and the binding of barstar.* J Mol Biol, 1997. **270**(1): p. 111-22.

65. **Gopalan V, Golbik R, Schreiber G, Fersht AR, Altman S,** *Fluorescence properties of a tryptophan residue in an aromatic core of the protein subunit of ribonuclease P from Escherichia coli.* J Mol Biol, 1997. **267**(1): p. 765-769.

66. **Schreiber, G. and A.R. Fersht,** *Rapid, electrostatically assisted association of proteins.* Nat Struct Biol, 1996. **3**(5): p. 427-31.

67. **Frisch, C., G. Schreiber, and A.R. Fersht,** *Characterization of in vitro oxidized barstar.* FEBS Lett, 1995. **370**(3): p. 273-7.

68. **Martinez, J.C., V.V. Filimonov, P.L. Mateo, G. Schreiber, and A.R. Fersht,** *A calorimetric study of the thermal stability of barstar and its interaction with barnase.* Biochemistry, 1995. **34**(15): p. 5224-33.

69. **Schreiber, G. and A.R. Fersht,** *Energetics of protein-protein interactions: analysis of the barnase-barstar interface by single mutations and double mutant cycles.* J Mol Biol, 1995. **248**(2): p. 478-86.

70. **Buckle, A.M., G. Schreiber, and A.R. Fersht,** *Protein-protein recognition: crystal structural analysis of a barnase-barstar complex at 2.0-A resolution.* Biochemistry, 1994. **33**(30): p. 8878-89.

71. **SCHREIBER G, RON EZ, GLASER G,** *PPGPP-MEDIATED REGULATION OF DNA-REPLICATION AND CELL-DIVISION IN ESCHERICHIA-COLI.* CURRENT MICROBIOLOGY, 1995. **30**(10): p. 27-32.

72. **AVIV M, GILADI H, SCHREIBER G, OPPENHEIM AB, GLASER G,** *EXPRESSION OF THE GENES-CODING FOR THE ESCHERICHIA-COLI INTEGRATION HOST FACTOR ARE CONTROLLED BY GROWTH-PHASE, RPOS, PPGPP AND BY AUTOREGULATION.* MOLECULAR MICROBIOLOGY, 1994. **14**(30): p. 1021-1031.

73. **Schreiber, G., A.M. Buckle, and A.R. Fersht,** *Stability and function: two constraints in the evolution of barstar and other proteins.* Structure, 1994. **2**(10): p. 945-51.

74. **Schreiber, G. and A.R. Fersht,** *Interaction of barnase with its polypeptide inhibitor barstar studied by protein engineering.* Biochemistry, 1993. **32**(19): p. 5145-50.

75. **Schreiber, G. and A.R. Fersht,** *The refolding of cis- and trans-peptidylprolyl isomers of barstar.* JBC, 1991. 266: p. 3760-3767.

76. **SCHREIBER G, METZGER S, AIZENMAN E, ROZA S, CASHEL M, GLASER G,** *OVEREXPRESSION OF THE RELA GENE IN ESCHERICHIA-COLI.* Biochemistry, 1993. 32(41): p. 11195-203.

77. **METZGER S, SCHREIBER G, AIZENMAN E, CASHEL M, GLASER G,** *CHARACTERIZATION OF THE RELA1 MUTATION AND A COMPARISON OF RELA1 WITH NEW RELA NULL ALLELES IN ESCHERICHIA-COLI.* JBC, 1989. 264: p. 21146-21152.

78. **METZGER S, BENDROR I, AIZENMAN E, SCHREIBER G, TOONE M, FRIESEN JD, CASHEL M, GLASER G,** *THE NUCLEOTIDE-SEQUENCE AND CHARACTERIZATION OF THE RELA GENE OF ESCHERICHIA-COLI.* JBC, 1988. 263: p. 15699-15704.

Book Chapters

1. **Schreiber, G.** (2001). Methods for Studying the Interaction of Barnase with Its Inhibitor Barstar, in Nuclease Methods and Protocols. C. H. Schein. New-York, Humana Press.

2. **Schreiber, G.**, (2002) Protein-protein interactions, in Biomolecular Sensors, E. Gizeli and R. Christopher, Editors. 2002, Taylor & Francis.

3. **Piehler, J. and Schreiber, G.** (2004, 2009) Free energy landscapes in protein-protein interactions, in Handbook of Cellular Signalling, Bradshaw, R, and Dennis, E. Editors, Academic Press.

4. **Schreiber, G.** (2009) The association of protein-protein complexes in Computational Protein-Protein Interactions, Nussinov R and Schreiber G. Editors, Taylor & Francis.

Books

• **Nussinov, R and Schreiber, G.** (2009) The association of protein-protein complexes .Editors, Taylor & Francis.

Appendix B

Curriculum Vitae
Prof. Gideon Schreiber

1980 - 1983:	B.Sc. *with distinction*, in food Technology from the Faculty of Agriculture, Hebrew University, Rehovot
1985 - 1987:	M.Sc. in Microbiology *with distinction* Thesis subject: Characterisation of *relA* mutations in *Escherichia coli*
1988 - 1991:	Doctoral studies, the Institute of Microbiology, Hebrew University, Hadassah Medical School, Jerusalem Supervisor: Prof. Gad Glaser Thesis subject: Studies of a global control system in prokaryotes mediated by the alarmon - ppGpp.
1992 - 1995:	Postdoctoral work at the Laboratory of Prof. Alan Fersht, MRC unit for protein function and design, Cambridge, UK. Subject: Intra and intermolecular interactions of barstar, the intercellular inhibitor of barnase from *Bacillus amyloliquefaciens*
1996 - 2002:	Senior Scientist, Department of Biological Chemistry, Weizmann Institute of Science
2002 - :	Associate Professor, Department of Biological Chemistry, Weizmann Institute of Science